

### **REMARKS**

Reconsideration of the pending application is respectfully requested.

Claims 32-48 are now pending, with claims 32 and 44 being independent.

Claims 15, 21 and 22 have been canceled without prejudice to or disclaimer of the subject matter recited therein.

Claims 32, 39, 41, 44 and 48 have been amended. In claim 32(a), the term "having farnesyltransferase activity" has been replaced with the term "comprising a farnesyltransferase beta subunit". In claim 32(b), the term "complement of the nucleotide sequence" has been replaced with the term "complement of the nucleotide sequence of (a)". In claims 39 and 41, the term "polynucleotide of Claim 32" has been replaced with the term "recombinant DNA construct of Claim 38". In claim 44, the term "having farnesyltransferase activity" has been replaced with the term "comprising a farnesyltransferase beta subunit". In both the preamble of claim 48 and in part 48(b), the term "farnesyltransferase" has been replaced with the term "farnesyltransferase beta subunit polypeptide". No new matter has been added.

Turning now to the Office Action mailed December 6, 2002:

As a preliminary matter, Applicants note that claims 15, 21 and 22 have been cancelled, and claims 32, 44 and 48 have been amended to recite the farnesyltransferase beta subunit, instead of the complete farnesyltransferase.

Regarding the Section 101 (utility) rejection, Applicants respectfully traverse and submit the following as rebuttal arguments for consideration:

First, the instant specification teaches an alpha subunit from corn, as well as the claimed beta subunit from corn, SEQ ID NO:12.

Second, Applicants submit that the beta subunit gene in a plant is not expected to be part of a large gene family, and Applicants are not aware of literature references that teach that there are many farnesyltransferases in plants.<sup>1</sup>

In the abstract of Yang et al. (1993) Plant Physiology 101:667-674 (cited in the specification, page 1, lines 23-25), the following statement occurs: "Gel blot hybridizations show that PsFTb is likely to be encoded by a single-copy gene and is

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<sup>1</sup> Applicants have found possible evidence of a second beta subunit gene in soybean. SEQ ID NO:15, encoding SEQ ID NO:16, represents a complete soybean beta subunit gene for farnesyltransferase. SEQ ID NO:17, encoding SEQ ID NO:18, is a partial gene sequence (single-pass EST sequence) for a beta subunit from soybean. SEQ ID NO:17 is 85.6% identical, using the Clustal V method of alignment, to SEQ ID NO:15. Amino acid 20 of SEQ ID NO:18 corresponds to the start methionine of SEQ ID NO:16. Amino acids 20-141 of SEQ ID NO:18 have 93.4% sequence identity with SEQ ID NO:16. SEQ ID NO:18 may represent a highly homologous additional beta subunit gene in soybean, or it may represent a different allele of the same genetic locus.

expressed as a transcript of approximately 1.7 kb." Thus, in pea, it appears that there is a single gene for the beta subunit of farnesyltransferase

In Arabidopsis, the ERA1 gene encodes the beta subunit of farnesyltransferase. Mutants in this gene were found to be devoid of farnesyltransferase activity in an in vitro assay (Cutler et al. (1996) Science 273:1239-1241), indicating a single gene in Arabidopsis for the the beta subunit of farnesyltransferase.<sup>2</sup>

Regarding the number of beta subunit genes in plants, Yalovsky et al. (2000) Plant Cell 12:1267-1278, write: "A single farnesyltransferase (FTase) and two geranylgeranyltransferases (GGTase-I and RabGGTase) have been identified in yeast, animals and plants. FTase and GGTase-I are heterodimeric enzymes with a common alpha subunit but distinct beta subunits that determine substrate specificity."

Third, Applicants submit that a beta subunit of farnesyltransferase has a well-recognized utility.

Applicants note the work of Pei et al. (1998) Science 282:287-290, cited on page 1, lines 33-37 of the instant specification, which indicate that plants with a decrease in farnesyltransferase (i.e., the beta subunit) should confer enhance tolerance to drought stress in plants. In the first paragraph (at the bottom of column one) of page 289 of Pei et al. (1998), the authors write: "After 12 days of drought treatment, WT plants showed severe wiltiness and chlorosis of rosette leaves. In contrast, *era1-2* plants were turgid and leaves remained green (Fig. 4A)." Under conditions of drought stress for WT (wild-type) plants, *era 1-2* plants (with a mutation in the ERA1 gene encoding the beta subunit of farnesyltransferase) showed tolerance to the drought stress.

Furthermore, Park et al. (1997) Science 275:1800-1804) have identified, in the X-ray crystal structure of rat farnesyltransferase, the three amino acids of the beta subunit that are involved in binding of the Zinc ion at the active site of the enzyme. These three Zn-binding amino acids are indicated in Appendices A1-A5, with arrows below the following amino acids: D (aspartate) at consensus residue 308, C (cysteine) at consensus residue 310, and H (histidine) at consensus residue 438.

The Arabidopsis amino acid sequence of the ERA1 gene of Pei et al. is believed to be partially represented by the 404-aa sequence, GI No. 1184953, of Cutler et al. and is believed to be substantially identical to the GI No. 8347240; the

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<sup>2</sup> The 404-amino acid Arabidopsis sequence of Cutler et al. (present in WO9906580-A2) was found to only represent part of the beta subunit sequence. The complete 482-amino acid Arabidopsis sequence was later determined by Ziegelhoffer et al. (2000) Proc. Natl. Acad. Sci. 97:7633-7638, and contains additional amino acids at the N-terminus.

only difference being at amino acid 129 (consensus amino acid 210 in Appendices A1-A5). This difference is not in any of the five regions of high sequence conservation discussed below. Additionally, a 404-aa partial sequence of the Arabidopsis ERA1 gene is available in patent application WO9906580-A2, published February 11, 1999. The amino acid sequence of WO9906580-A2 differs from GI No. 1184953 at amino acids 129, 325 and 344.

Applicants attach hereto on Appendices A1-A5<sup>3</sup> an amino acid sequence alignment of the following: the complete amino acid sequence of the beta subunit gene from corn (SEQ ID NO:12 of the pending claims) and soybean (SEQ ID NO:16), partial amino acid sequences for the beta subunit gene from rice (SEQ ID NO:14) and a possible second beta subunit gene from soybean (SEQ ID NO:18), and amino acid sequences encoded by the beta subunit genes from pea (GI No. 169049), tomato (GI No. 1815668), Arabidopsis (GI No. 1184953; 404-aa partial sequence), Arabidopsis (GI No. 8347240; 482-aa complete sequence) and rat (GI No. 266753).

Appendix B sets forth the percent identities and percent divergences, based on the Clustal method of alignment using default parameters, among each of the sequences of Appendices A1-A5 to the others.

Both SEQ ID NO:12 of the pending claims and GI No. 1184953 (i.e., what is believed to be the basis of Pei et al.) contain these three Zn-binding amino acids.

Additionally, five regions of high sequence conservation are indicated above the sequences on Appendices A1-A5 by the letters A through E. The five regions of high homology are described in the following table:

Table 1. Regions of High Sequence Conservation within the Beta Subunit

Region	Position (in Consensus)	Length in Amino Acids	# of Identical Residues	% Identity
A	111-117	7	7	100%
B	153-169	17	14	82.4%
C	257-268	12	11	91.7%
D	301-315	18	16	88.9%
E	428-446	19	15	78.9%

In view of the foregoing, Applicants respectfully request withdrawal of the Section 101 (utility) rejection.

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<sup>3</sup> In Appendices A1-A5, the numbering of the consensus sequence is given below the sequences, and the numbering of individual sequences is given to the left of each row of sequence, and to the right of the final row of sequence. Bases that are identical in all genes at a given position are indicated by an asterisk.

Regarding the Section 112, 1<sup>st</sup> paragraph rejection that one skilled in the art would not know how to use the claimed invention, Applicants refer to the above remarks in response to the Section 101 (utility) rejection. Withdrawal of this Section 112, 1<sup>st</sup> paragraph rejection is respectfully requested.

Regarding the Section 112, 1<sup>st</sup> paragraph enablement rejection, Applicants respectfully traverse.

As discussed above with respect to Park et al., knowledge about conserved domains and active site residues, present in sequences from the art and the specification, provide specific guidance to one of ordinary skill as to which structures are likely to have enzyme activity. The experimentation necessary to determine activity is not undue in this field, where the level of skill is very high. Molecular biological techniques are available from the prior art to either isolate sequences closely related to SEQ ID NO:12 from other plant sources, or to make changes in the sequence of SEQ ID NO:12 that would not eliminate beta subunit function. In this regard, the specification teaches both the alpha and beta subunit genes from corn. These genes can be expressed in a heterologous organism, such as E. coli, and assayed for farnesyltransferase activity in vitro according to methods known in the art, such as the method of Qian et al. (1996) Plant Cell 8:2381-2394.

In view of the foregoing, Applicants respectfully request withdrawal of the Section 112, 1<sup>st</sup> paragraph enablement rejection.

Finally, regarding the Section 112, 2<sup>nd</sup> paragraph rejections, Applicants submit that these rejections have been rendered either moot (cancelled claims 15 and 21-22) or inapplicable in view of the claim amendments. Accordingly, Applicants respectfully request withdrawal of this rejection.

Applicants believe that this Amendment and Response to Office Action is fully responsive to each of the points raised in the Office Action. Allowance of the above-referenced application is respectfully requested.

Applicants have also filed simultaneously herewith a Supplemental Information Disclosure Statement citing references discussed herein which have not been previously cited and/or considered.

Please charge any fees or credit any overpayment of fees which are required in connection herewith to Deposit Account No. 04-1928 (E. I. du Pont de Nemours and Company).

Applicants' undersigned may be reached at the below-listed numbers.

Respectfully submitted,



J. KENNETH JOUNG  
Attorney For Applicants  
Registration No. 41,881  
Telephone: 302-992-4929  
Facsimile: 302-892-1026

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Attachments: Appendices A1-A5  
Appendix B

601

	A
	***** *
SEQ ID NO:12 corn	38 ARVGDIYRSLFGAAPNTKSIMLELWRDQHIEYLTPGLRHMGPAFHVLDANRPWLCYWMVH
SEQ ID NO:14 rice	45 AKVGEIYRVLEGNAPNANSIMLELRQHQVEYLTGRLKHLGPSFHVL DANRPWCYWI IH
SEQ ID NO:16 soy	4 SQVFQIYQLFATIPRNAQTLMLELQRDNHMQYVSKGLRHLSSAFSVLDANRPWLCYWI FH
SEQ ID NO:18 soy	23 SQVFQIYQLFATIPGSAQNLMLELQRDNHMQYLSKGLRHLSSAFSVLDANRPWLCYWI FH
gi169049 pea	24 SQVFHIYQLFANIPNAQSII-----RPWLCYWI IH
gi1815668 tomato	18 RRVREIYDYFYISISPNSPSDL IEIERDKHFYLSQGLRKLGPSFVLDASRPWLCYWL H
gi1184953 Arab	2 -----EIQRDKQLDYL MKGLRQLGPQFS SLDANRPWLCYWI H
gi8347240 Arab	58 NDVFGIYN YFDASDVSTQKYMEIQRDQLDYL MKGLRQLGPQFS SLDANRPWLCYWI L H
gi266753 rat	53 EKIQEVEFSSYKFENHLVPR--LV LQREKHFFHYLL KGLRQLTDAYECCLDASRPWLCYWI L H

61  
120

APPENDIX A2

B

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SEQ ID NO:12 corn      98 PLALDEALDDLENDLIDFLARCQDKDGGYSGPGQLPHLATTTAAVNTLVTIGSERAL
SEQ ID NO:14 rice     105 ALALDE-IPDDVEDDIVDFLSRCQDKDGGYSGPGQLPHLATTTAAVNTLVTIGSERAL
SEQ ID NO:16 soy       64 SIALSGESVDELEDNAIDFLNRCQDPNGGYAGGPGQMPHIATTTAAVNSLITLGEKSL
SEQ ID NO:18 soy       83 SIALGESVDELEDNITIDFLNRCQDPNGGYAGGPGQMPHIATTTAAVNTLITLGGQKS-
gi169049 pea          55 SIALGESIDDDLEDNTVDFLNRCQDPNGGYAGGPGQMPHIATTTAAVNTLITLGEKSL
gi1815668 tomato      78 SIALGESIGKLENDALIDFLTRCQDKDGGYSGGPGQMPHIATTTAAVNSLITLGPKEAL
gi1184953 Arab         40 SIALGETVDELESNALIDFLGRCQSGEGYGGGPGQLPHLATTTAAVNALVTLGGDKAL
gi8347240 Arab        118 SIALGETVDELESNALIDFLGRCQSGEGYGGGPGQLPHLATTTAAVNALVTLGGDKAL
gi266753 rat          110 SLELDEPIPIQIVATDVQCQFLELCQSPDGGFGGPGQYPHLAPTTAAVNALCITGTEEAY
121
180
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SEQ ID NO:12 corn      158 SSINRGNLYNFMLQMKDVSAGAFRMDGGEIDVRASYTAISVASLVNILLDFKLAKGVGYI
SEQ ID NO:14 rice     164 SSVNRDNLKFMRLMKDTSAGAFRMDGGEIDVRASYTAISVASLVNILLDELAKGVGNYI
SEQ ID NO:16 soy       124 ASINRDKLYGFLRRMKQPNNGGFRMHDEGEIDVRACYTAISVASLVNILLDELIQNVGDIYI
SEQ ID NO:18 soy       141 -----
gi169049 pea          115 ASINRNKLYGFMRMRMKQPNNGGFRMHDEGEIDVRACYTAISVASLVNILLDELIKNVGDFI
gi1815668 tomato      138 SSINREKLYTFLLRMKDASGGFRMDGGEVDVRACYTAISVANILNIVDELIGHVGNYI
gi1184953 Arab         100 SSINREKMSCFLRRMKDTSGGFRMHDMGEIDVRACYTAISVASILNIMDELITQGLGDIYI
gi8347240 Arab         178 SSINREKMSCFLRRMKDTSGGFRMHDMGEMDVRACTAISVASILNIMDELITQGLGDIYI
gi266753 rat          170 NVINREKLLQYLYSLKQPDGSFLMHVGGEVDVRSAYCAASVASLTNIIITPDLFEGTAEWI
181
240
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## APPENDIX A3

\*\*\* \*\* C \*\*\*

SEQ ID NO:12 corn 218 ARCQTYEGGIAGEPYAEAHGGYTFCGLAALLILNEAEKVDLPSLIGWVAFRQGV-ECGFQ  
SEQ ID NO:14 rice 224 TRCQTYEGGIAGEPYAEAHGGYTFCGLATMILLNEVDKLDIASLIGWVAFRQGV-ECGFQ  
SEQ ID NO:16 soy 184 ISCQTYEGGIAGEPGESEAHGGYTFCGLATMILLGEVNHLDLPRLVDWVFRQGV-ECGFQ  
SEQ ID NO:18 soy 141 -----  
g1169049 pea 175 LSCQTYEGGILAGEPGESEAHGGYTFCGLAAMILLIGEVRDLDPRLDWVFRQGV-ECGFQ  
g11815668 tomato 198 LSCQTYEGGIAGEPGESEAHGGYTFCGLAAMILLINEVDRLDLPGLIDWVFRQGV-EGGFQ  
g11184953 Arab 160 LSCQTYEGGIAGEPGESEAHGGYTCGLAAMILLINEVDRLNLDLSLMNAVHRQGV-EMGFQ  
g18347240 Arab 238 LSCQTYEGGIGGEPGESEAHGGYTCGLAAMILLINEVDRLNLDLSLMNAVHRQGV-EMGFQ  
g1266753 rat 230 ARCQNWEGGIGGVPGEAHGGYTFCGLAALVILKKERSLNLSLLQWVTSRQMRFEQGFQ  
241 300

## D

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SEQ ID NO:12 corn 277 GRTNKLVDGCYSFWQGAIAIAFTOKLITIVDKQL-----KSSYS  
SEQ ID NO:14 rice 283 GRTNKLVDGCYSFWQGAIALIT-----  
SEQ ID NO:16 soy 243 GRTNKLVDGCYSFWQGAVALLORLSSIINKQMEETSQIFAVS-----YVSEAKESLD  
SEQ ID NO:18 soy 141 -----  
g1169049 pea 234 GRTNKLVDGCYSFWQGAVALLORLHSIIDEQMAEASQFVTVS-----DAPEKECLD  
g11815668 tomato 257 GRTNKLVDGCYSFWQGAVALLORLNLIVHEQLGLSNDLSTESADDSSELSDEEHLE  
g11184953 Arab 219 GRTNKLVDGCYTFWQAPCVLLQRLYSTNDHDVHGSSHI-----SEGTNEEHHAHDEDDLE  
g18347240 Arab 297 GRTNKLVDGCYTFWQAPCVLLQRLYSTNDHDVHGSSHI-----SEGTNEEHHAHDEDDLE  
g1266753 rat 290 GRCNKLVDGCYSFWQAGLLPLLHR-----ALHAQGDPAALS  
301 ↑ ↑ 360



## APPENDIX A4

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SEQ ID NO:12 corn      315 CKRPSGEDACSTSSYGCTAKKSSSAVDYAKFGFDFTIQSNQIGPLFHNIALQQYILLCSQ
SEQ ID NO:14 rice      305 -----VHRVAP-----
SEQ ID NO:16 soy       296 GTSSHA--TCRGEHEGT--SESSSSDFKNIAKYFINEWRAQEPLEFHSIALQQYILLCAQ
SEQ ID NO:18 soy       141 -----
gi169049 pea          287 GTSSHA--TSHIRHEGM--NESCSDVKNIGYNFISEWRQSEPLFHSIALQQYILLCSQ
gi1815668 tomato      317 GISSHVQDTFPLGQAGACQENASHSPKADTGYEFINRP IAMRPLFDSMYLQQYVLLCSQ
gi1184953 Arab        275 DSDDD-----DSDDEDNDEDSVNGHRIHHTSTYINRRMQL--VFDSLGLQRYVLLCSK
gi8347240 Arab        353 DSDDD-----DSDDEDNDEDSVNGHRIHHTSTYINRRMQL--VFDSLGLQRYVLLCSK
gi266753 rat          325 -----MSHW-----MFHQALQRYILMCCQ
                                     361                                     420
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E

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** *** * * * *
SEQ ID NO:12 corn      375 VLEGGLRDKPGKNRDHYHSCYCLSGLAVSQYSAMTDTGSCPLPQHVLGPYSN-LLEPIHP
SEQ ID NO:14 rice      311 -----
SEQ ID NO:16 soy       351 EQEGGLRDKPGKRRDHYHTCYCLSLCQYSWSKHPDSPPLPNLVLPYSN-LLEPIHP
SEQ ID NO:18 soy       141 -----
gi169049 pea          342 EQDGLRDKPGKRRDHYHSCYCLSLCQYSWSKRPDSPPLPKVVMGPYSN-LLEPIHP
gi1815668 tomato      377 IEVGGFRDKPGKGRDYHTCYCLSGLSIAQYSWTDADSTPLPRDVFGPYSKCLLEQVHP
gi1184953 Arab        326 IPDGGFRDKPRKPRDYHTCYCLSGLSVAQHAWLKDEDTPPLTRDIMGYSN-LLEPVQL
gi8347240 Arab        404 IPDGGFRDKPRKPRDYHTCYCLSGLSVAQHAWLKDEDTPPLTRDIMGYSN-LLEPVQL
gi266753 rat          345 CPAGGLDKPGKSRDYHTCYCLSGLSIAQHF----GSGAMLHDVVMGVPENVLQPTHP
                                     421                                     480
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APPENDIX A5

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SEQ ID NO:12 corn	434	LYNVVLDR-YHTAYEF-----	-FSEE	452
SEQ ID NO:14 rice	311	-----	-TAK	313
SEQ ID NO:16 soy	410	LENVVLGR-YREAHEF-----	-FTES	429
SEQ ID NO:18 soy	141	-----	-----	141
gi169049 pea	401	LENVVLDR-YREAHEF-----	-F-----	SQL
gi1815668 tomato	437	LENVVLDR-YYEAREY-----	-SQACETVSPSLAPTFSET	470
gi1184953 Arab	385	LHNIVMDQ-YNEAIEF-----	-F-----	-FKAA
gi8347240 Arab	463	LHNIVMDQ-YNEAIEF-----	-F-----	-FKAA
gi266753 rat	400	VYNIQPDKVIQATTHFLQKPVPGFECECD---	-AVTSDPATD	437
	481			522

